PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 5/10, C07K 14/705, 16/28, C12Q 1/68, G01N 33/50, 33/566, A61K 31/70, A01K 67/027

A1

(11) International Publication Number: WO 99/02675

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/BE98/00108

(22) International Filing Date: 9 July 1998 (09.07.98)

(30) Priority Data:
97870101.9 9 July 1997 (09.07.97) EP

(71) Applicant *(for all designated States except US)*: EURO-SCREEN S.A. [BE/BE]; Avenue des Becassines 7, B–1160 Bruxelles (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: COMMUNI, Didier [BE/BE]; Groendallaan 19, B–1800 Vilvoorde (BE). BOEYNAEMS, Jean–Marie [BE/BE]; Avenue Peter Benoît 5, B–1780 Wemmel (BE).

(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B–1083 Brussels (BE).

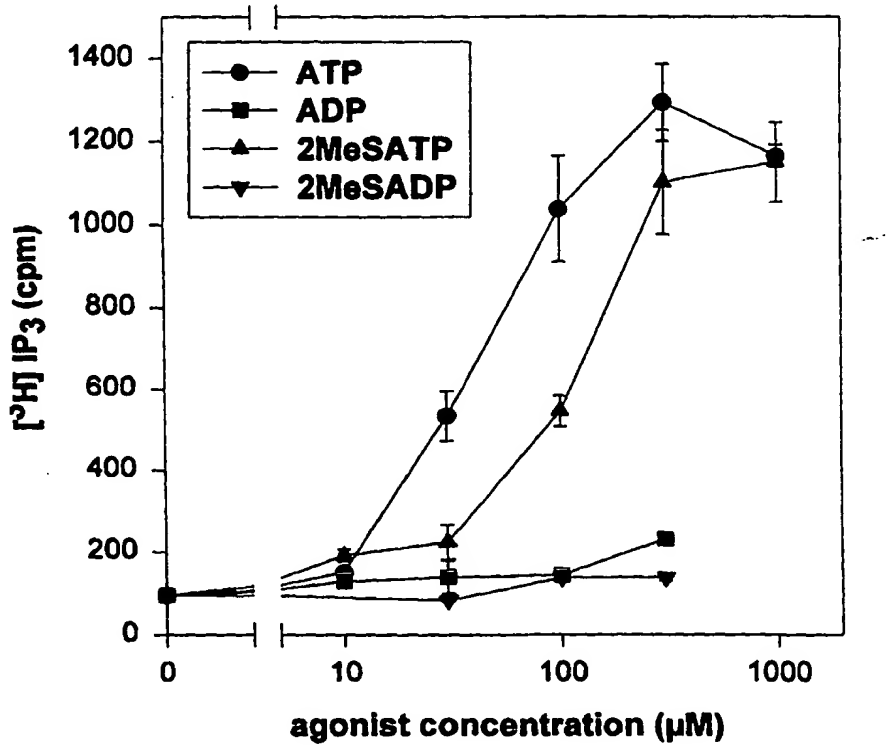
(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: G–COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATP

(57) Abstract

The present invention concerns a G–coupled receptor which has an amino acid sequence having more than 50 % homology with the amino acid sequence shown in Figure 1.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

G-COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATP

Object of the present invention

The present invention concerns a new G protein-coupled receptor having selective affinity for ATP and the nucleic acid molecule encoding said receptor, vectors comprising said nucleic acid molecule, cells transformed by said vector, antibodies directed against said receptor, nucleic acid probes directed against said nucleic acid molecule, pharmaceutical compositions comprising said products and non human transgenic animals expressing the receptor according to the invention or the nucleic acid molecule according to said receptor.

Background of the invention

An impressive number of P2 receptors subtypes has been cloned since 1993. A new molecular nomenclature has then been created in which G protein-coupled P2 receptors have been named P2Y while P2 receptors having an intrinsic ion channel activity have been named P2X. The P2Y family encompasses selective purinoceptors (the $P2Y_1$ receptor activated by ATP and ADP), nucleotide receptors responsive to both adenine and uracil nucleotides ($P2Y_2$ receptor: activated equipotentally by ATP and UTP) and pyrimidinoceptors (the $P2Y_3$ and $P2Y_6$ receptors activated by UDP; the $P2Y_4$ receptor: activated by UTP). The

$P2Y_5$ and $P2Y_7$ receptors display limited homologies with the other members of the P2Y family. They have been included in this family especially on the basis of radioligand binding studies showing affinities for adenine nucleotides (1-18).

Summary of the invention

The present invention concerns a new receptor having the amino acids sequence of figure 1 or any receptor which presents more than 50%, preferably more than 70%, more preferably more than 85%, more specifically more than 95% homology with the amino acids sequence of figure 1.

The present invention concerns also the receptor having at least the amino acids sequence of figure 1 or a portion thereof, preferably an amino acids sequence wherein the large extracellular part (the NH_2 portion of 450 amino acids sequence the end of which (♦) is represented on Fig. 1) has been truncated or active parts of said portion such as the sixth and seventh transmembrane domains comprising the amino acids : His^{686}, Arg^{689} and Arg^{728}.

The present invention is also related to said NH_2 portion of 450 amino acids sequence, including peptides reproducing or mimicking a portion of this sequence or of organic molecules sharing the effects of these peptides.

Indeed, the inventors have discovered that either the whole receptor having the amino acids sequence of figure 1 or its portion (preferably the amino acids sequence wherein the large extracellular part of 450 amino acids has been truncated and starting from (♦) in Fig. 1) seems to have the same industrial application (said portion will be identified hereafter as the P2Y11 receptor or sequence).

The first industrial application of this receptor or its portions is the screening of agonists and

antagonists of said receptor which may have advantageous pharmaceutical or diagnostical properties. The second industrial application of the receptor according to the invention or of its portions or of active parts of its portions is the identification of patients who may present genetic disorders induced by an inactive receptor or by an inactive portion of said receptor.

According to a preferred embodiment of the present invention, said receptor is a human receptor.

ATP seems to be the preferential natural agonist of this receptor : UTP, UDP, AP_4A, AP_6A, AMP and adenosine seem to be unable to stimulate the phosphoinositide pathway or were much less potent that ATP.

Therefore, the invention is also related to a new G-coupled receptor, its portions or active parts of its portions having a selective affinity for ATP. "A selective affinity for ATP" means that ATP is able to induce the formation of a functional response (preferably the accumulation of Inositol triphosphate IP_3 and a rise of intracellular Ca^{2+}) in a short time of incubation with said agonist (preferably in less than 5 min, more preferably less than 1 min) while the other known agonists of P2Y (UTP, UDP, AP_4A, AP_6A, AMP and adenosine were unable to stimulate said receptor or were much less potent than ATP and induce a detectable functional response by said receptor.

The present invention is also related to a nucleic acid molecule, such as a DNA molecule or an RNA molecule, encoding the receptor, its portions or active parts of its portions according to the invention.

Preferably, said DNA molecule is a cDNA molecule or a genomic DNA molecule.

Preferably, said nucleic acid molecule has more than 50%, preferably more than 70%, more preferably

more than 85%, more specifically more than 95% homology with the DNA sequence shown in figure 1.

Preferably, the invention is related to a nucleic acid molecule which has more than 50%, preferably more than 70%, more preferably more than 85%, more specifically more than 95% homology with this DNA sequence (shown in the figure 1), wherein the DNA sequence encoding the 450 amino acids of the NH_2 portion were truncated.

The present invention is also related to the vector comprising the nucleic acid molecule according to the invention. Preferably, said vector is adapted for expression in a cell and comprises the regulatory elements necessary for expressing the amino acid molecule in said cell operatively linked to the nucleic acid sequence according to the invention as to permit expression thereof.

Preferably, said cell is selected from the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells. The vector according to the invention is a plasmid or a virus, preferably a baculovirus, an adenovirus or a semliki forest virus.

The present invention concerns also the cell transformed by the vector according to the invention, said cell is preferably non-neuronal in origin and is selected from the group consisting of a COS-7 cell, a CHO cell, an LM(tk-) cell, an NIH-3T3 cell or a 1321N1 astrocytoma cell.

The present invention is also related to a nucleic acid probe comprising the nucleic acid molecule according to the invention, of at least 15 nucleotides capable of specifically hybridising with a unique sequence included in the sequence of the nucleic acid molecule encoding the receptor according to the invention. Said nucleic acid probe may be a DNA or an RNA molecule.

The invention concerns also an antisense oligonucleotide having a sequence capable of specifically hybridising to an mRNA molecule encoding the receptor according to the invention so as to prevent translation of

said mRNA molecule or an antisense oligonucleotide having a sequence capable of specifically hybridising to the cDNA molecule encoding the receptor according to the invention.

Said antisense oligonucleotide may comprise chemical analogs of nucleotide or substances which inactivate mRNA, or be included in an RNA molecule endowed with ribozyme activity.

Another aspect of the present invention concerns a ligand (preferably an antibody) other than known molecules, especially the ATP, capable of binding to the receptor according to the invention and an anti-ligand (preferably also an antibody) capable of competitively inhibiting the binding of said ligand to the receptor according to the invention.

Preferably, said antibody is a monoclonal antibody directed to an epitope of the receptor according to the invention and present on the surface of a cell expressing said receptor.

The invention concerns also the pharmaceutical composition comprising an effective amount of oligonucleotide according to the invention, effective to decrease the activity of said receptor by passing through a cell membrane and binding specifically with mRNA encoding the receptor according to the invention in the cell so as to prevent its translation. The pharmaceutical composition comprises also a pharmaceutically acceptable carrier capable of passing through said cell membrane.

Preferably, in said pharmaceutical composition, the oligonucleotide is coupled to a substance, such as a ribozyme, which inactivates mRNA.

Preferably, the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure. The structure of the pharmaceutically acceptable carrier in said pharmaceutical composition is

capable of binding to a receptor which is specific for a selected cell type.

Preferably, said pharmaceutical composition comprises an amount of the antibody according to the invention effective to block the binding of a ligand to the receptor according to the invention and a pharmaceutically acceptable carrier.

The present invention concerns also a transgenic non human mammal overexpressing (or expressing ectopically) the nucleic acid molecule encoding the receptor according to the invention.

The present invention also concerns a transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to the invention.

According to a preferred embodiment of the invention, the transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid according to the invention is so placed as to be transcripted into antisense mRNA which is complementary to the mRNA encoding the receptor according to the invention and which hybridises to mRNA encoding said receptor, thereby reducing its translation. Preferably, the transgenic non human mammal according to the invention comprises a nucleic acid molecule encoding the receptor according to the invention and comprises additionally an inducible promoter or a tissue specific regulatory element.

Preferably, the transgenic non human mammal is a mouse.

The invention relates also to a method for determining whether a ligand as an agonist or an antagonist of the receptor according to the invention can be specifically bound to said receptor; said method comprising the steps of contacting a cell or a cell extract from cells transfected with a vector according to the invention and expressing the nucleic acid molecule encoding said

receptor, possibly isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction or with the cell under conditions permitting binding of said ligand to the receptor and detecting, possibly by means of a bioassay such as a modification in the second messenger concentration or a modification in the cellular metabolism (preferably determined by the acidification rate of the culture medium), an increase in the receptor activity), thereby determining whether the ligand binds to the receptor, possibly as an agonist or as an antagonist of said receptor.

Preferably, the second messenger assay comprises measurement of intracellular cAMP, intracellular inositol phosphate (IP3), intracellular diacylglycerol (DAG) concentration or intracellular calcium mobilisation.

Preferably, the cell used in said method is a mammalian cell non neuronal in origin, such as a COS-7 cell, a CHO cell, a LM(tk-) cell an NIH-3T3 cell or 1321N1 cell. In said method, the ligand is not previously known.

The invention is also related to the ligand isolated and detected by any of the preceding methods.

The present invention concerns also the pharmaceutical composition which comprises an effective amount of an agonist or an antagonist of the receptor according to the invention, effective to reduce the activity of said receptor and a pharmaceutically acceptable carrier.

The P2Y11 transcripts (obtained from the nucleotidic sequence starting from (♦) in Fig. 1) are detectable in HL-60 human leukaemia cells. Expression of P2Y11 receptor mRNA is increased by agents (ripnoic acid, DMSO) known to induce the granulocytic differenciations of HL-60 cells. However, the P2Y11 transcripts could not be detected in mature neutrophils. Therefore, a first industrial application of the product according to the invention is the diagnostic of leukaemia, preferably by

Northern blot analysis using the nucleotidic sequence encoding the P2Y11 receptor according to the invention.

The present invention is also related to a diagnostic device or kit comprising the elements for the diagnostic of specific leukaemia, preferably HL-60 human leukaemia, comprising the receptor according to the invention, the nucleic acid sequence encoding said receptor, a nucleic acid probe comprising the nucleic acid molecule according to the invention of at least 15 nucleotides capable of specifically hybridising with a unique sequence included in the sequence of the nucleic acid molecule encoding the receptor according to the invention, such as an antisense oligonucleotide or a ligand such as an antibody, preferably a monoclonal antibody, capable of binding or competitively inhibiting the binding of a ligand to the receptor according to the invention. Said diagnostic device or kit could be used for the specific diagnostic or for the monitoring of the evolution of tumoral cells, especially leukaemia HL-60 cells.

Therefore, the previously described methods may be used for the screening of drugs (having advantageously anti-tumoral properties) which specifically bind to the receptor according to the invention.

Another industrial application of the present invention is related to the use of said drugs, preferably ligands or anti-ligands according to the invention, for the prevention and/or the treatment of specific diseases such as neutropenie or agranulocytose infections or cancer.

The invention is also related to the drugs isolated and detected by any of these methods.

The present invention concerns also a pharmaceutical composition comprising said drugs and a pharmaceutically acceptable carrier.

The invention is also related to a method of detecting expression of a receptor according to the invention by detecting the presence of mRNA coding for a

receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to the invention under hybridising conditions and detecting the presence of mRNA hybridised to the probe, thereby detecting the expression of the receptor by the cell.

The hybridisation conditions above-described are preferably standard stringent conditions as described by Sambrook et al. (§9.47-9.51 in Molecular Cloning : Laboratory Manual, Cold Spring Harbour, Laboratory Press, New York (1989)).

The present invention concerns also a method for diagnosing a predisposition to a disorder associated with the activity of the receptor according to the invention. Said method comprises:

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridising to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridised to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose

thereby predisposition to the disorder if the patterns are the same.

A last aspect of the present invention concerns a method of preparing the receptor according to the invention, which comprises:

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

Brief description of the drawings

Figure 1 represents the nucleotide and deduced amino acid sequence of the new human P2Y receptor. The putative phosphorylation sites by protein kinase C or by calmodulin-dependent protein kinases are indicated respectively by a black circle (●) or a black diamond (◆). The potential N-glycosylation site is indicated by a black square (■).

Figure 2 represents dendrogram of the structural relatedness of the $P2Y_{11}$ receptor with the other P2Y subtypes. The plot was constructed using the multiple sequence alignment program Pileup of the GCG package. The $P2Y_5$-like published sequence (18) is identical to the $P2Y_9$ sequence submitted to the GenBank/EMBL Data Bank.

Figure 3 represents Northern blot analysis of $P2Y_{11}$ messenger expression. Each lane of the MTN blot contains 2 µg of polyA$^+$ RNA from several human tissues. Each lane of the HL-60 blot contains 10 µg of total RNA from differentiated or undifferentiated HL-60 cells. Hybridization with the probe was performed as described under Materials and Methods. The pictures of the MTNII blot and the HL-60 blot were obtained respectively, from an autoradiography and from a PhosphorImager SI (Molecular Dynamics). The 2kb-length $P2Y_{11}$ transcripts are indicated by a black arrow.

Figure 4 represents concentration-action curves of several nucleotides on IP_3 and cAMP accumulation in cells transfected with the $P2Y_{11}$ receptor. 1321N1 and CHO-K1 transfected cells were assayed for the accumulation of, respectively, IP_3 (A) or cAMP (B) in response to various concentrations of the following nucleotides: ATP, 2MeSATP, ADP and 2MeSADP. Incubation times were 30 s for IP_3 measurements and 15 min for cAMP assays. The data represent the means ± S.D. of triplicate experimental points and are representative of two independent experiments.

Description of a preferred embodiment of the present invention

Experimental Procedures

Materials

Trypsin was from Flow Laboratories (Bioggio, Switzerland). Culture media, G418, fetal calf serum (FCS), restriction enzymes and Taq polymerase were purchased from GIBCO BRL (Grand Island, NY). The radioactive products myo-D-[2-^{3}H]inositol (17.7 Ci/mmol) and [α^{32}P]ATP (800 Ci/mmol) were from Amersham (Ghent, Belgium). Dowex AG1X8 (formate form) was from Bio-Rad Laboratories (Richmond, Calif.). ATP, ADP, AMP, adenosine, UTP, UDP, AP_4A, AP_6A, all-trans

retinoic acid (RA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-methylthio-ATP (2MeSATP), 2-methylthio-ADP (2MeSADP) and 8 (p-sulfophenyl) theophylline were from Research Biochemicals International (Natick, MA). Forskolin was purchased from Calbiochem (Bierges, Belgium). Indomethacin and dimethyl sulfoxide (DMSO) were from Merck (Netherlands). Rolipram was obtained from the Laboratoires Jacques Logeais (Trappes, France). The HL-60 human cell line was obtained from the American Type Culture Collection (Rockville, USA). The human genomic DNA library was from Stratagene (La Jolla, CA). pEFIN3 is an expression vector obtained from Euroscreen (Brussels, Belgium). Multiple Human Tissues Northern blot (MTN) were from Clontech (Palo Alto, CA).

Cloning and sequencing

A human placenta cDNA library was screened at moderate stringency with an [α^{32}P] dATP labelled P2Y$_4$ receptor probe corresponding to a partial sequence covering the third to the seventh transmembrane segments. Three overlapping clones encoding a new G protein-coupled receptor were isolated, but did not contain the 3' end of the coding region. A human genomic DNA library was then screened with this partial sequence to obtain the complete sequence of this new receptor. The hybridization conditions for screening the two libraries were 6 X SSC (1 X SSC: 0.15 M NaCl, 0.015 M sodium citrate) and 40% formamide at 42 °C for 14 hours and the final washing conditions were 0.5 X SSC, 0.1% SDS at 60 °C. Four genomic clones were purified and shown to contain the 3' end of the open reading frame missing in the cDNA clones. The sequence was obtained on both strands after subcloning of overlapping restriction fragments in M13mp18 and M13mp19 using the Sanger dideoxy nucleotide chain termination method.

Northern blot analysis

Two blots of human organs (MTN I and MTN II: 2 µg polyA$^+$ RNA/lane) and a blot containing total RNA from differentiated and undifferentiated HL-60 cells (10 µg of total RNA/lane) were hybridized with a probe corresponding to the new receptor in order to characterize its tissue distribution. The HL-60 cells were maintained in RPMI 1640 supplemented with 10% FCS, 5 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin at 37 °C with 5% CO_2. The HL-60 cells were incubated during six days with or without 1 µM retinoic acid or 1.25% DMSO or during eight hours with 25 nM TPA. The RNA from the differentiated or undifferentiated HL-60 cells was prepared with the RNeasy kit (Quiagen). The blots were prehybridized 8 hours at 42 °C in a 50% formamide, 2% SDS solution and hybridized for 18 hours in the same solution supplemented with the [α^{32}P] labelled probe. The final washing conditions were 0.1 X SSC and 0.1% SDS at 55 °C. The blots were exposed during twelve days and visualized as an autoradiography or using the PhosphorImager SI (Molecular Dynamics).

Cell culture and transfection

The complete sequence of the new receptor according to the invention was subcloned between the Hind III and Nhe I sites of the bicistronic pEFIN3 expression vector. 1321N1 and CHO-K1 cells were transfected with the recombinant pEFIN3 plasmid or with the plasmid alone using the calcium phosphate precipitation method as described (19). The transfected cells were selected with 400 µg/ml G418 in complete medium (10% FCS, 100 units/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin B in Dulbecco's modified Eagle's medium (DMEM)) two days after transfection and maintained in the same medium (10).

Inositol phosphates (IP) measurement

1321N1 cells were labelled for 24 hours with 10 mCi/ml [^{3}H] inositol in inositol free DMEM containing 5% FCS, antibiotics, amphotericin, sodium pyruvate and 400 µg/ml G418. Cells were washed twice with Krebs-Ringer Hepes (KRH) buffer of the following composition (124 mM NaCl, 5 mM KCl, 1.25 mM $MgSO_4$, 1.45 mM $CaCl_2$, 1.25 mM KH_2PO_4, 25 mM Hepes (pH:7.4) and 8 mM glucose) and incubated in the same medium for 30 min. The cells were then challenged by various nucleotides for 30 s. The incubation was stopped by the addition of an ice cold 3% perchloric acid solution. IP were extracted and separated on Dowex columns as previously described (20).

Cyclic AMP measurements

Stably transfected CHO-K1 or 1321N1 cell lines were spread on Petri dishes (150.000 cells per dish) and cultured in Ham's F12 or DMEM medium containing 10% FCS, antibiotics, amphotericin, sodium pyruvate and 400 µg/ml G418. Cells were preincubated for 30 min in KRH buffer with rolipram (25 µM) and incubated for different times in the presence of the agonists (15 min in most experiments). The incubation was stopped by the addition of 1 ml HCl 0.1 M. The incubation medium was dried up, resuspended in water and diluted as required. Cyclic AMP was quantified by radioimmunoassay after acetylation as previously described (21).

Results

Cloning and Sequencing

A human cDNA placenta library was screened at moderate stringency with a human $P2Y_4$ probe. Nine clones which hybridized weakly with the $P2Y_4$ probe were obtained, purified and analyzed. Six of them corresponded to the

sequence of the $P2Y_6$ receptor (10) while three overlapping clones corresponded to a partial sequence encoding a new G protein-coupled receptor, displaying about 30% identity with the other P2Y receptors. The partial open reading frame started with an ATG-codon in a Kozak consensus but the 3′ end was missing in all three cDNA clones. The Inventors screened a human genomic DNA library using this partial sequence as a probe. Four overlapping genomic clones were obtained. Mapping of the coding sequence and partial sequencing allowed to determine that the gene encoding the new receptor contains an intron interrupting the coding sequence at the 5' end of the gene. This intron separates the three first codons from the rest of the coding sequence. Beside these first codons, the four genomic clones contained the complete open reading frame including the 3′ end missing in the cDNA clones. The full open reading frame appeared as 1113 base pairs (bp) long and encoded a protein of 371 amino acids containing one potential site for N-linked glycosylation and two potential sites for phosphorylation by protein kinase C or calmodulin-dependent protein kinases (Fig. 1). The new receptor, provisionally named $P2Y_{11}$, displays significant homologies with the other P2Y receptors (Fig. 2). In particular, 33% and 28 % amino acid identity were observed respectively with the human $P2Y_1$ and $P2Y_2$ receptors.

Tissue distribution of the new receptor

The tissue distribution of the new receptor transcripts was investigated by Northern blotting (Fig. 3) by using a probe corresponding to a partial sequence encoding transmembrane segments 3 to 7. The strongest signal was observed for human spleen and corresponded to a 2 kilobase (kb)-length messenger RNA (MTN II). A weaker signal was observed in small intestine (MTN II). All the lanes in MTN I (heart, brain, placenta, lung, liver,

skeletal muscle, kidney, pancreas) were negative. The Inventors also detected specific 2 kb-length transcripts in HL-60 cells. The signal was very weak in the undifferentiated HL-60 cells but increased when the cells had been treated with retinoic acid or DMSO. No increase was observed when the HL-60 cells were stimulated with TPA. A weak non-specific hybridization with 18S rRNA was observed. These data were confirmed with a non-overlapping probe corresponding to the first 300 bp of the coding region, presenting limited homologies with the other P2Y subtypes.

Functional Expression of the new receptor in 1321N1 astrocytoma cells

The complete sequence of the new receptor was introduced in the pEFIN3 expression vector in order to transfect the 1321N1 astrocytoma cell line, used previously to characterize several P2Y subtypes (6, 10, 12). The pool of G418-resistant clones was tested for its functional response to several nucleotides. ATP (100 µM) induced a strong inositol trisphosphate (IP_3) accumulation in cells transfected with the recombinant plasmid, whereas ADP, AMP, adenosine, UTP, UDP, AP_4A and AP_6A were inactive at the same concentration. All nucleotides were totally inactive on the cells transfected with the vector alone. We then tested ATP, 2MeSATP, ADP and 2MeSADP in a large range of concentrations. As shown in Fig. 4A, ATP was the most potent agonist (EC_{50} ATP = 38 ± 7 µM; EC_{50} 2MeSATP = 118 ± 15 µM; means ± range of two independent experiments). The effect of ADP and 2MeSADP were minimal. Pertussis toxin (50 ng/ml; 24 h pretreatment) had no effect on the ATP response, whereas a lower concentration of pertussis toxin was previously shown to abolish the response to UTP in $P2Y_4$ transfected 1321N1 astrocytoma cells (22). A response to ATP (10 µM) was also obtained following $[Ca^{2+}]_i$

measurements performed on the 1321N1 transfected cells while ADP was inactive at this concentration.

Functional expression of the new receptor in CHO-K1 cells

The 1321N1 cells transfected with the new receptor displayed a strong cAMP increase in response to ATP. A much lower but significant endogeneous response due to the degradation of adenine nucleotides into adenosine was also obtained in the 1321N1 cells transfected with the vector alone. The CHO-K1 cells express an endogeneous $P2Y_2$ receptor coupled to the phosphoinositide pathway (23) but do not possess adenosine receptors coupled to adenylyl cyclase. We therefore used CHO-K1 cells in order to characterize the coupling of the new receptor to the cAMP pathway. A pool of G418 resistant CHO-K1 clones was first tested for its response to several nucleotides at a concentration of 100 µM. ATP was able to induce a strong increase in the cAMP content, whereas it was inactive on cells transfected with the vector alone. ADP, AMP, adenosine, UTP and UDP were completely inactive. Concentration-action curves were established for ATP, 2MeSATP, ADP and 2MeSADP (Fig. 4B). The rank order of potency was the same as in the inositol phosphate study on 1321N1 cells. The curves were obtained after 15 min of stimulation by the agonists; however a significant cAMP response to ATP was already obtained after 2 min of stimulation. The response to ATP (30 µM) was inhibited neither by indomethacin (10 µg/ml, present from 30 minutes before the stimulation and readded in the stimulation medium) nor by 8 (p-sulfophenyl) theophylline (100 µM).

The receptor according to the invention presents some structural peculiarities which differentiate it from some other P2Y subtypes. Concerning its gene structure, the coding sequence is interrupted by an intron. Comparison between the cDNA and the genomic DNA sequences has clearly demonstrated the absence of intron in the

coding region of the human $P2Y_1$ receptor (24, 25), the rat $P2Y_2$ receptor (26) and the rat $P2Y_6$ receptor (11). In terms of protein structure, the second and third extracellular loops are significantly longer than those of the other P2Y receptors. The homology with the other subtypes is relatively weak (about 30%). The closest G-coupled receptor is the human $P2Y_1$ receptor (33%) which is also a receptor responsive to adenine nucleotides (3, 4). Mutagenesis experiments with the $P2Y_2$ receptor have identified three positively charged amino acids in the sixth and seventh transmembrane domains (His^{262}, Arg^{265} and Arg^{292}), which play a crucial role in nucleotide binding (presumably by neutralizing the negative charge of the phosphate groups) (27). These three residues are conserved in this new receptor.

So far, eight P2Y receptor subtypes are described in the literature ($P2Y_1$-$P2Y_8$). In addition, two sequences related to the $P2Y_5$ receptor and named $P2Y_9$ and $P2Y_{10}$, have been recently submitted to the GenBank/EMBL Data Bank. The $P2Y_9$ sequence is identical to that recently published under the name "$P2Y_5$-like" (18). Therefore the new receptor described in this paper might be called $P2Y_{11}$. However, it is already clear that the nomenclature needs a revision. It was recently demonstrated that the $P2Y_7$ receptor is actually a receptor for leukotriene B_4 (16) and there is no functional evidence that the $P2Y_5$ and related receptors ($P2Y_5$-like or $P2Y_9$, $P2Y_{10}$) are nucleotide receptors (17, 18).

Among the sixteen human organs tested by Northern blotting, $P2Y_{11}$ transcripts of 2 kb size were only detectable in spleen, and with lower intensity in small intestine. This distribution is reminiscent of that of the human $P2Y_6$ 1.7 kb-messenger. The observation of the

expression of the $P2Y_{11}$ receptor in the HL-60 cell line shows that this expression was strongly increased following treatment by DMSO or retinoic acid, two agents known to induce the differentiation of these cells into granulocytes (28). On the contrary, TPA, which is known to induce the monocytic differentiation of the HL-60 cells (29), did not stimulate the expression of the $P2Y_{11}$ receptor. The confirmation of these data with a second probe of the $P2Y_{11}$ cDNA, that shares little similarity with other P2Y sequences, excludes possible cross-hybridization with another P2Y receptor transcript. In view of the Northern blots results, it is tempting to speculate that the $P2Y_{11}$ receptor is involved in the recently described accumulation of cAMP in ATP-stimulated HL-60 cells (30).

Among the P2Y receptors, the $P2Y_{11}$ subtype has the unique property to activate both the phosphoinositide and the cAMP pathways. Other cloned P2Y receptors are coupled to phospholipase C exclusively. The rank order of potency of agonists was the same for the two pathways. ATP was clearly much more potent than ADP. This difference may be even underestimated as a result of low level ATP contamination in ADP preparation or conversion of ADP into ATP during assays (4, 11). On the other hand, 2MeSATP had the same maximal effect than ATP but presented a lower potency, while 2MeSADP, a potent activator of the $P2Y_1$ and P_{2T} subtypes (4), was almost inactive. The EC_{50} values were comparable to those obtained in the study concerning the effects of extracellular nucleotides on the cAMP accumulation in the HL-60 cells (30).

Stimulatory effects of adenine nucleotides on the cAMP pathway have been described in different cell types (31, 32). In most cases, the stimulatory effect of nucleotides was inhibited by xanthines. These data suffer from the fact that it is difficult to exclude that the effect of adenine nucleotides is mediated by their

degradation into adenosine due to the ubiquitous presence of ectonucleotidases expressed at the cell surface. The cAMP study has been performed with CHO-K1 cells to avoid the endogeneous cAMP response to adenosine in the astrocytoma cell line. Neither in untransfected CHO-K1 cells nor in $P2Y_{11}$-transfected CHO-K1 cells did adenosine increase cAMP accumulation. Furthermore the cAMP response to ATP was insensitive to xanthine inhibition. It was also insensitive to indomethacin, indicating that is not mediated by the release of prostaglandins. It is unlikely that the cAMP response would be an indirect consequence of the calcium response since the use of ATP, which activates the phosphoinositide pathway by the activation of $P2Y_2$ endogeneous receptors, or the use of calcium ionophores in the CHO-K1 cells failed to stimulate cAMP accumulation (33). Therefore these data constitute the first strong evidence that a P_2 receptor can be coupled to the stimulation of adenylyl cyclase.

REFERENCES

1. Abbrachio, M.P. and Burnstock, G. (1994) *Pharmacol. Ther.* **64**, 445-475.
2. Fredholm, B.B. et al. (1997) *Trends Pharmacol. Sci.* **18**, 79-82.
3. Webb, T.E. et al. (1993) *FEBS Lett.* **324**, 219-225.
4. Léon, C. et al. (1997) *FEBS Lett.* **403**, 26-30.
5. Lustig, K.D. et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5113-5117.
6. Parr, C.E.et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3275-3279.
7. Bogdanov, Y. et al. (1997) *J. Biol. Chem.* **272**, 12583-12590.
8. Webb, T.E. et al. (1996) *Mol. Pharmacol.* **50**, 258-265.
9. Chang, K. et al. (1995) *J. Biol. Chem.* **270**, 26152-26158.
10. Communi, D. et al; (1996) *Biochem. Biophys. Res. Commun.* **222**, 303-308.
11. Nicholas, R.A. et al. (1996) *Mol. Pharmacol.* **50**, 224-229.
12. Communi, D. et al. (1995) *J. Biol. Chem.* **270**, 30849-30852.
13. Nguyen, T. et al. (1995) *J. Biol. Chem.* **270**, 30845-30848.
14. Webb, T.E. et al. (1996) *Biochem. Biophys. Res. Commun.* **219**, 105-110.
15. Akbar, G.K.M. et al. (1996) *J. Biol. Chem.* **271**, 18363-18367.
16. Yokomizo, T. et al; (1997) *Nature* **387**, 620-624.
17. Schachter, J.B. and Harden, T.K. (1997) *Br. J. Pharmacol.* **121**, 338-344.
18. Janssens, R. et al. (1997) *Biochem. Biophys. Res. Commun.* **226**, 106-112.

19. Velu, T.J. et al. (1989) *J. Cell. Biochem.* **39**, 153-166.

20. Communi, D. et al. (1995) *Circ. Res.* **76**, 191-198.

21. Brooker, G. et al. (1979) *Adv. Cyclic Nucleotide Res.* **10**, 1-33.

22. Communi, D. et al. (1996) *Eur. J. Pharmacol.* **317**, 383-389.

23. Iredale, P.A. and Hill, S.J. (1993) *Br. J. Pharmacol.* **110**, 1305-1310.

24. Schachter, J.B. et al. (1996) *Br. J. Pharmacol.* **118**, 167-173.

25. Janssens, R. et al. (1996) *Biochem. Biophys. Res. Commun.* **221**, 588-593.

26. Godecke, S. et al. (1996) *Am. J. Physiol.* **270**, C570-C577.

27. Erb, L. et al. (1995) *J. Biol. Chem.* **270**, 4185-4188.

28. Breitman, T.R. et al. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2936-2940.

29. Blaineau, C. et al. (1983) *Embo J.* **11**, 2007-2012.

30. Jiang, L. et al. (1997) *Biochem. Biophys. Res. Commun.* **232**, 626-630.

31. Matsuoka, I. et al. (1995) *Mol. Pharmacol.* **47**, 855-862.

32. Song, S.L. and Chueh, S.H. (1996) *Brain Res.* **734**, 243-251.

33. Thekkumkara, T.J. et al. (1995) *Mol. Cell Biochem.* **152**, 77-86.

CLAIMS.

1. Receptor which has an amino acid sequence having more than 50% homology with the amino acid sequence shown in Figure 1.

2. Receptor according to claim 1, which has at least the amino acid sequence shown in Figure 1 or a portion thereof.

3. Receptor according to claim 1 or 2 having a selective affinity ADP.

4. Receptor according to any of the preceding claims, belonging to the P2Y receptor family.

5. Receptor according to any of the preceding claims, being a G protein-coupled receptor.

6. Receptor according to any of the preceding claims, being a human receptor.

7. Nucleic acid molecule encoding the receptor according to any of the preceding claims.

8. Nucleic acid molecule according to claim 7, wherein the nucleic acid molecule is DNA or RNA molecule.

9. DNA molecule according to claim 8, which is a cDNA molecule or a genomic DNA molecule.

10. Nucleic acid molecule according to any of the claims 7 to 9, having more than 50% homology to the DNA sequence shown in Figure 1.

11. DNA molecule according to claim 10, which has at least the DNA sequence as shown in figure 1 or a portion thereof.

12. Vector comprising the nucleic acid molecule according to any of the claims 7 to 11.

13. Vector according to claim 12, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic

acid molecule according to any of the claims 7 to 11 as to permit expression thereof.

14. Vector of claim 13, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

15. Vector according to any of the claims 12 to 14, wherein the vector is a plasmid or a virus, preferably a baculovirus, an adenovirus or a Semliki Forest virus.

16. Cell comprising the vector according to any of the claims 12 to 15.

17. Cell of claim 16, wherein the cell is a mammalian cell, preferably non neuronal in origin.

18. Cell of claim 16, wherein the cell is selected from the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 astrocytoma cells.

19. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridising with a unique sequence included within the nucleic acid molecule according to any of the claims 11 to 15.

20. Nucleic acid probe of claim 19, wherein the nucleic acid is DNA or RNA.

21. Antisense oligonucleotide having a sequence capable of specifically hybridising to a mRNA molecule of claim 8, so as to prevent translation of the mRNA molecule.

22. Antisense oligonucleotide having a sequence capable of specifically hybridising to the DNA molecule of claim 9.

23. Antisense oligonucleotide according to claim 21 or 22, comprising chemical analogs of nucleotides.

24. Ligand other than purine and pyridine nucleotides capable of binding to a receptor according to any of the claims 1 to 6.

25. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 24 to the receptor according to any of the claims 1 to 6.

26. Ligand according to claim 24, which is an antibody.

27. Anti-ligand according to claim 25, which is an antibody.

28. Antibody according to claim 26 or 27, which is a monoclonal antibody.

29. Monoclonal antibody according to claim 28, directed to an epitope of the receptor according to any of the claims 1 to 6, present on the surface of a cell expressing said receptor.

30. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 21, effective to decrease activity of the receptor according to any of the claims 1 to 6 by passing through a cell membrane and binding specifically with mRNA encoding said receptor in the cell so as to prevent its translation, and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

31. Pharmaceutical composition of claim 30, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

32. Pharmaceutical composition of claim 31, wherein the substance which inactivates mRNA is a ribozyme.

33. Pharmaceutical composition according to any of the claims 30 to 32, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

34. Pharmaceutical composition of claim 33, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

35. Pharmaceutical composition which comprises an effective amount of the anti-ligand of claim 30, effective to block binding of a ligand to the receptor according to any of the claims 1 to 6 and a pharmaceutically acceptable carrier.

36. Transgenic non human mammal expressing the nucleic acid molecule according to any of the claims 7 to 11.

37. Transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to any of the claims 1 to 6.

38. Transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid molecule according to any of the claims 7 to 11 so placed as to be transcripted into antisense mRNA which is complementary to the mRNA of claim 8 and which hybridises to said mRNA thereby reducing its translation.

39. Transgenic non human mammal according to any of the claims 36 to 38, wherein the nucleic acid according to any of the claims 7 to 11 additionally comprises an inducible promoter.

40. Transgenic non human mammal according to any of the claims 36 to 39, wherein the nucleic acid according to claim 7 to 11 additionally comprises tissue specific regulatory elements.

41. Transgenic non human mammal according to any of the claims 36 to 40, which is a mouse.

42. Method for determining whether a ligand can specifically bind to a receptor according to any of the claims 1 to 6, possibly as an agonist or an antagonist of said receptor; said method comprising the steps of :

contacting a cell or cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, possibly isolating a membrane fraction from the cell extract, with the ligand under conditions permitting binding of said ligand to said receptor, possibly by the activation of a functional response, and detecting the presence of any such ligand bound specifically to said receptor, possibly by means of a bioassay such as a modification of the production of a second messenger or an increasing in the receptor activity, thereby determining whether the ligand binds specifically to said receptor, possibly as an agonist or an antagonist of said receptor.

43. A method according to claim 42, wherein the second messenger assay comprises measurement of intracellular cAMP, intracellular Inositol phosphate, intracellular diacylglycerol concentration or intracellular calcium mobilisation.

44. Method according to claim 42 or 43, wherein the cell is a mammalian cell, preferably non neuronal in origin, and selected from the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

45. Method according to any of the preceding claims 42 to 44, wherein the ligand is not previously known.

46. Ligand detected by the method according to any of the preceding claims 42 to 45.

47. Pharmaceutical composition which comprises the ligand according to claim 46 and a pharmaceutically acceptable carrier.

48. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 6 on the surface of the cell, which comprises contacting a cell transfected with a vector

expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting binding of said drugs to the receptor, and determining those drugs which specifically bind to the transfected cell, thereby identifying drugs which specifically bind to the receptor.

49. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 6 on the surface of the cell, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cells extract, contacting the membrane fraction with a plurality of drugs and determining those drugs which bind to the transfected cell, thereby identifying drugs which specifically bind to said receptor.

50. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor agonists.

51. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which

activate such receptor using a bio-assay, such as a modification in a second messenger concentration, thereby identifying drugs which act as receptor agonists.

52. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor antagonists.

53. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger concentration, thereby identifying drugs which act as receptor antagonists.

54. Drug detected by any of the methods according to claims 48 to 53.

55. Pharmaceutical composition comprising a drug according to claim 54.

56. Method of detecting the expression of the receptor according to any of the claims 1 to 6, by detecting the presence of mRNA coding said receptor, which

comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to claim 19 under hybridising conditions, and detecting the presence of mRNA hybridised to the probe, thereby detecting the expression of the receptor by the cell.

57. Method of detecting the presence of the receptor according to any of the claims 1 to 6 on the surface of a cell, which comprises contacting the cell with the antibody of claim 26 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

58. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 6, which comprises producing a transgenic non human mammal according to any of the claims 36 to 41 whose levels of receptor expression are varied by use of an inducible promoter which regulates the receptor expression.

59. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 6, which comprises producing a panel of transgenic non human mammals according to any of the claims 36 to 41, each expressing a different amount of said receptor.

60. Method for identifying an antagonist of the receptor according to any of the claims 1 to 6 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of the receptor, which comprises administering the antagonist to a transgenic non human mammal according to any of the claims 36 to 41 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the

transgenic non human mammal as a result of receptor activity, thereby identifying the antagonist.

61. Antagonist identified by the method of claim 60.

62. Pharmaceutical composition comprising an antagonist according to claim 61 and a pharmaceutically acceptable carrier.

63. Method for identifying an agonist of the receptor according to any of the claims 1 to 6 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of said receptor, which comprises administering the agonist to a transgenic non human mammal according to any of the claims 36 to 41 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal, the alleviation of the abnormalities indicating the identification of the agonist.

64. Agonist identified by the method of claim 63.

65. Pharmaceutical composition comprising an agonist according to claim 64 and a pharmaceutically acceptable carrier.

66. Method for diagnosing a predisposition to a disorder associated with the activity of a specific allele of the receptor according to any of the claims 1 to 6, which comprises:

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridising to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridised to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

67. Method of preparing the purified receptor according to any of the claims 1 to 6, which comprises:

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

68. Use of the pharmaceutical composition according to claim 47, 55, 62 or 65, in the preparation of a medicament for the treatment and/or the prevention of the neutropenie, agranulocytose infections or cancer.

$P2Y_{11}$ sequence

FIG.1a

```
   1                                                   M  G  Q  S  G  R  S      20
   1 GAATTCGGCACGAGGAGGCCTCGTGGAGGACACAGCAGCATGGGACAGTCAGGGAGGTCC             60

  21  R  H  Q  K  R  A  R  A  Q  A  Q  L  R  N  L  E  A  Y  A  A              40
  61 CGGCACCAGAAGCGCGCCCGCGCCCAGGCGCAGCTCCGCAACCTCGAGGCCTATGCCGCG             120

  41  N  P  H  S  F  V  F  T  R  G  C  T  G  R  N  I  R  Q  L  S              60
 121 AACCCGCACTCGTTCGTGTTCACGCGAGGCTGCACGGGTCGCAACATCCGGCAGCTCAGC             180

  61  L  D  V  R  R  V  M  E  P  L  T  A  S  R  L  Q  V  R  K  K              80
 181 CTGGACGTGCGGCGGGTCATGGAGCCGCTCACTGCCAGCCGTCTGCAGGTTCGTAAGAAG             240

  81  N  S  L  K  D  C  V  A  V  A  G  P  L  G  V  T  H  F  L  I             100
 241 AACTCGCTGAAGGACTGCGTGGCAGTGGCTGGGCCCCTCGGGGTCACACACTTTCTGATC             300

 101  L  S  K  T  E  T  N  V  Y  F  K  L  M  R  L  P  G  G  P  T             120
 301 CTGAGCAAAACAGAGACCAATGTCTACTTTAAGCTGATGCGCCTCCCAGGAGGCCCCACC             360

 121  L  T  F  Q  V  K  K  Y  S  L  V  R  D  V  V  S  S  L  R  R             140
 361 TTGACCTTCCAGGTGAAGAAGTACTCGCTGGTGCGTGATGTGGTCTCCTCACTGCGCCGG             420

 141  H  R  M  H  E  Q  Q  F  A  H  P  P  L  L  V  L  N  S  F  G             160
 421 CACCGCATGCACGAGCAGCAGTTTGCCCACCCACCCCTCCTGGTACTCAACAGCTTTGGC             480

 161  P  H  G  M  H  V  K  L  M  A  T  M  F  Q  N  L  F  P  S  I             180
 481 CCCCATGGTATGCATGTGAAGCTCATGGCCACCATGTTCCAGAACCTGTTCCCCTCCATC             540

 181  N  V  H  K  V  N  L  N  T  I  K  R  C  L  L  I  D  Y  N  P             200
 541 AACGTGCACAAGGTGAACCTGAACACCATCAAGCGCTGCCTCCTCATCGACTACAACCCC             600

 201  D  S  Q  E  L  D  F  R  H  Y  S  I  K  V  V  P  V  G  A  S             220
 601 GACTCCCAGGAGCTGGACTTCCGCCACTATAGCATCAAAGTTGTTCCTGTGGGCGCGAGT             660

 221  R  G  M  K  K  L  L  Q  E  K  F  P  N  M  S  R  L  Q  D  I             240
 661 CGCGGGATGAAGAAGCTGCTCCAGGAGAAGTTCCCCAACATGAGCCGCCTGCAGGACATC             720

 241  S  E  L  L  A  T  G  A  G  L  S  E  S  E  A  E  P  D  G  D             260
 721 AGCGAGCTGCTGGCCACGGGCGCGGGGCTGTCGGAGAGCGAGGCAGAGCCTGACGGCGAC             780

 261  H  N  I  T  E  L  P  Q  A  V  A  G  R  G  N  M  R  A  Q  Q             280
 781 CACAACATCACAGAGCTGCCTCAGGCTGTCGCTGGCCGTGGCAACATGCGGGCCCAGCAG             840

 281  S  A  V  R  L  T  E  I  G  P  R  M  T  L  Q  L  I  K  V  Q             300
 841 AGTGCAGTGCGGCTCACCGAGATCGGCCCGCGGATGACACTGCAGCTCATCAAGGTCCAG             900

 301  E  G  V  G  E  G  K  V  M  F  H  S  F  V  S  K  T  E  E  E             320
 901 GAGGGCGTCGGGGAGGGCAAAGTGATGTTCCACAGTTTTGTGAGCAAGACGGAGGAGGAG             960

 321  L  Q  A  I  L  E  A  K  E  K  K  L  R  L  M  A  Q  R  Q  A             340
 961 CTGCAGGCCATCCTGGAAGCCAAGGAGAAGAAGCTGCGGCTGATGGCGCAGAGGCAGGCC            1020

 341  Q  Q  A  Q  N  V  Q  R  K  Q  E  Q  R  E  A  H  R  K  K  S             360
1021 CAGCAGGCCCAGAATGTGCAGCGCAAGCAGGAGCAGCGGGAGGCTCACAGAAAGAAGAGC            1080
```

FIG. 1b

```
361   L  E  G  M  K  K  A  R  V  G  G  S  D  E  E  A  S  G  I  P   380
1081  CTGGAGGGCATGAAGAAGGCACGGGTCGGGGGTAGTGATGAAGAGGCCTCTGGGATCCCT  1140

381   S  R  T  A  S  L  E  L  G  E  D  D  D  E  Q  E  D  D  D  I   400
1141  TCAAGGACGGCGAGCCTGGAGTTGGGTGAGGACGATGATGAACAGGAAGATGATGACATC  1200

401   E  Y  F  C  Q  A  V  G  E  A  P  S  E  D  L  F  P  E  A  K   420
1201  GAGTATTTCTGCCAGGCGGTGGGCGAGGCGCCCAGTGAGGACCTGTTCCCCGAGGCCAAG  1260

421   Q  K  R  L  A  K  S  P  G  R  K  R  K  R  W  E  M  D  R  G   440
1261  CAGAAACGGCTTGCCAAGTCTCCAGGGCGGAAGCGGAAGCGGTGGGAAATGGATCGAGGT  1320

441   A  K  S  C  P  A  N  F  L  A  A  A  D  D  K  L  S  G  F  Q   460
1321  GCCAAGTCCTGCCCTGCCAACTTCTTGGCAGCTGCCGACGACAAACTCAGTGGGTTCCAG  1380

461   G  D  F  L  W  P  I  L  V  V  E  F  L  V  A  V  A  S  N  G   480
1381  GGGGACTTCCTGTGGCCCATACTGGTGGTTGAGTTCCTGGTGGCCGTGGCCAGCAATGGC  1440

481   L  A  L  Y  R  F  S  I  R  K  Q  R  P  W  H  P  A  V  V  F   500
1441  CTGGCCCTGTACCGCTTCAGCATCCGGAAGCAGCGCCCATGGCACCCCGCCGTGGTCTTC  1500

501   S  V  Q  L  A  V  S  D  L  L  C  A  L  T  L  P  P  L  A  A   520
1501  TCTGTCCAGCTGGCAGTCAGCGACCTGCTCTGCGCTCTGACGCTGCCCCCGCTGGCCGCC  1560

521   Y  L  Y  P  P  K  H  W  R  Y  G  E  A  A  C  R  L  E  R  F   540
1561  TACCTCTATCCCCCCAAGCACTGGCGCTATGGGGAGGCCGCGTGCCGCCTGGAGCGCTTC  1620

541   L  F  T  C  N  L  L  G  S  V  I  F  I  T  C  I  S  L  N  R   560
1621  CTCTTCACCTGCAACCTGCTGGGCAGCGTCATCTTCATCACCTGCATCAGCCTCAACCGC  1680

561   Y  L  G  I  V  H  P  F  F  A  R  S  H  L  R  P  K  H  A  W   580
1681  TACCTGGGCATCGTGCACCCCTTCTTCGCCCGAAGCCACCTGCGACCCAAGCACGCCTGG  1740

581   A  V  S  A  A  G  W  V  L  A  A  L  L  A  M  P  T  L  S  F   600
1741  GCCGTGAGCGCTGCCGGCTGGGTCCTGGCCGCCCTGCTGGCCATGCCCACACTCAGCTTC  1800

601   S  H  L  K  R  P  P  Q  Q  G  A  G  N  C  S  V  A  R  P  E   620
1801  TCCCACCTGAAGAGGCCGCCGCAGCAGGGGGCGGGCAACTGCAGCGTGGCCAGGCCCGAG  1860

621   A  C  I  K  C  L  G  T  A  D  H  G  L  A  A  Y  R  A  Y  S   640
1861  GCCTGCATCAAGTGTCTGGGGACAGCAGACCACGGGCTGGCGGCCTACAGAGCGTATAGC  1920

641   L  V  L  A  G  L  G  C  G  L  P  L  L  L  T  L  A  A  Y  G   660
1921  CTGGTGCTGGCGGGGGTTGGGCTGCGGCCTGCCGCTGCTGCTCACGCTGGCAGCCTACGGC  1980

661   A  L  G  R  A  V  L  R  S  P  G  M  T  V  A  E  K  L  R  V   680
1981  GCCCTCGGGCGGGCCGTGCTACGCAGCCCAGGCATGACTGTGGCCGAGAAGCTGCGTGTG  2040

681   A  A  L  V  A  S  G  V  A  L  Y  A  S  S  Y  V  P  Y  H  I   700
2041  GCAGCGTTGGTGGCCAGTGGTGTGGCCCTCTACGCCAGCTCCTATGTGCCCTACCACATC  2100

701   M  R  V  L  N  V  D  A  R  R  R  W  S  T  R  C  P  S  F  A   720
2101  ATGCGGGTGCTCAACGTGGATGCTCGGCGGCGCTGGAGCACCCGCTGCCCGAGCTTTGCA  2160

721   D  I  A  Q  A  T  A  A  L  E  L  G  P  Y  V  G  Y  Q  V  M   740
2161  GACATAGCCCAGGCCACAGCAGCCCTGGAGCTGGGGCCCTACGTGGGCTACCAGGTGATG  2220
```

```
 741   R  G  L  M  P  L  A  F  C  V  H  P  L  L  Y  M  A  A  V  P     760
2221   CGGGGCCTCATGCCCCTGGCCTTCTGTGTCCACCCTCTACTCTACATGGCCGCAGTGCCC  2280

 761   S  L  G  C  C  C  R  H  C  P  G  Y  R  D  S  W  N  P  E  D     780
2281   AGCCTGGGCTGCTGCTGCCGACACTGCCCCGGCTACAGGGACAGCTGGAACCCAGAGGAC  2340

 781   A  K  S  T  G  Q  A  L  P  L  N  A  T  A  A  P  K  P  S  E     800
2341   GCCAAGAGCACTGGCCAAGCCCTGCCCCTCAATGCCACAGCCGCCCCTAAACCGTCAGAG  2400
                                  ◆
 801   P  Q  S  R  E  L  S  Q  *
2401   CCCCAGTCCCGTGAGCTGAGCCAATGA
```

FIG.1c

FIG.2

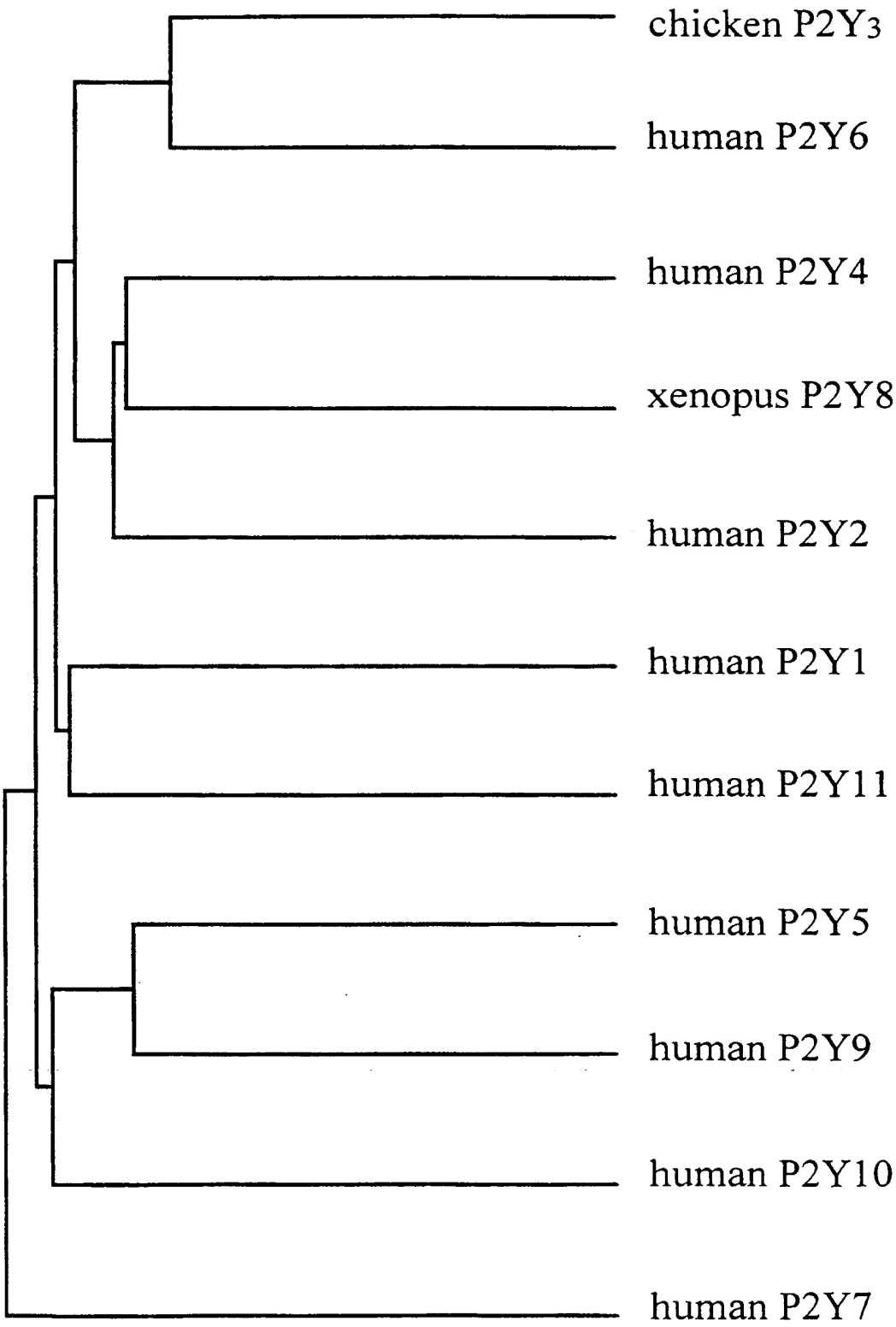
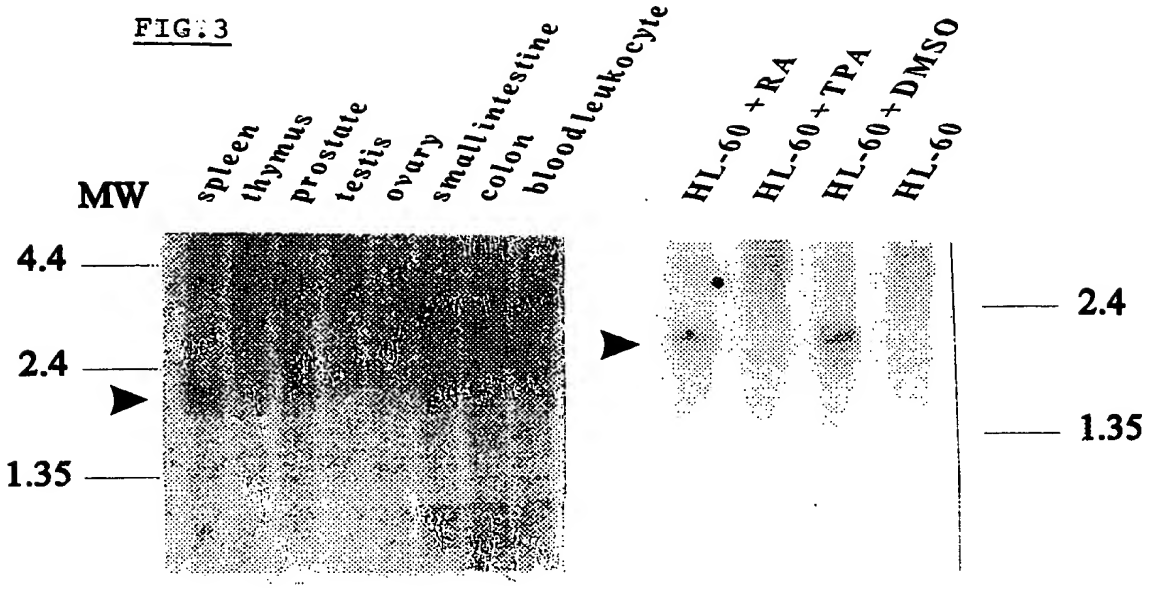


FIG.3



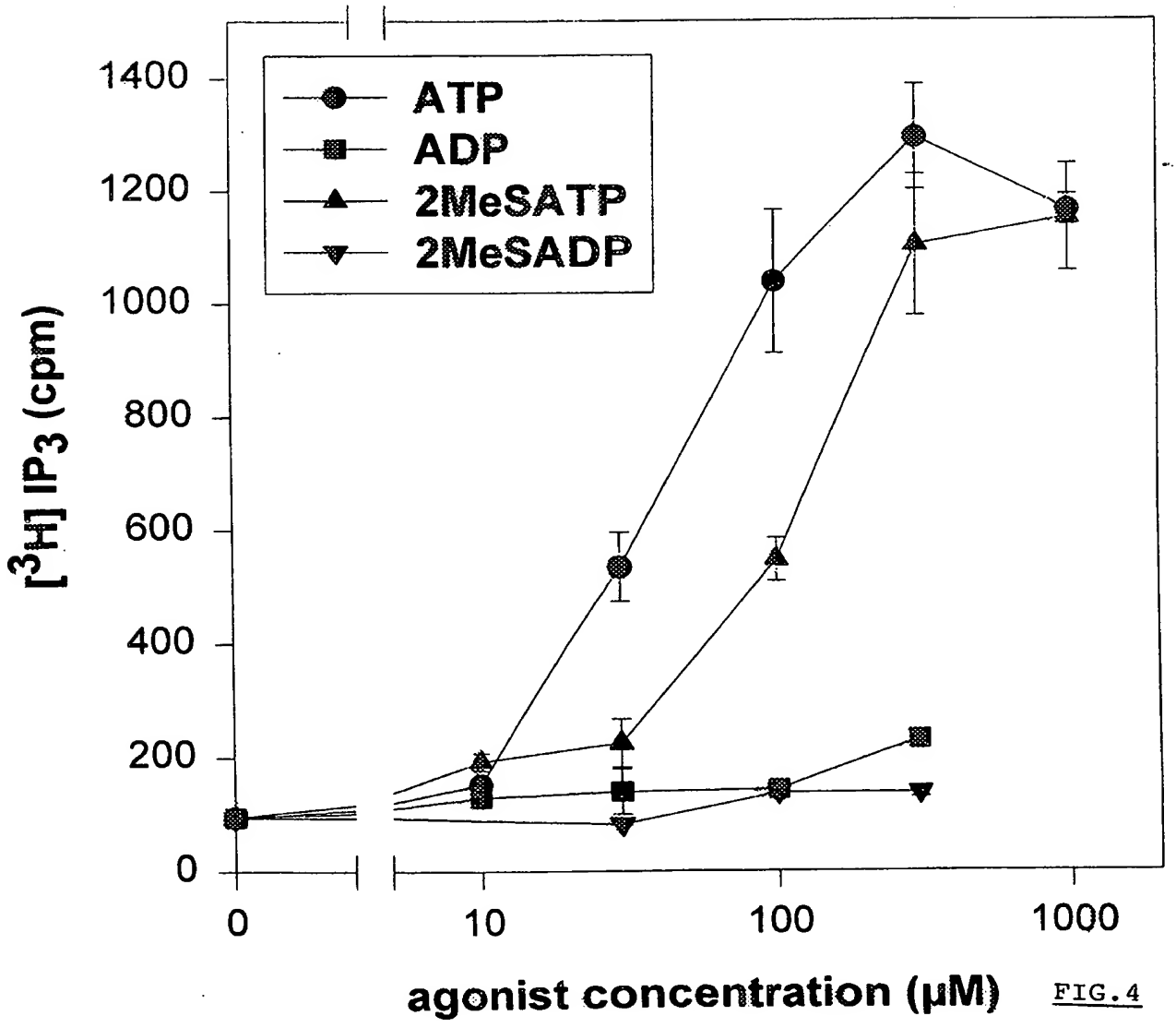


FIG.4

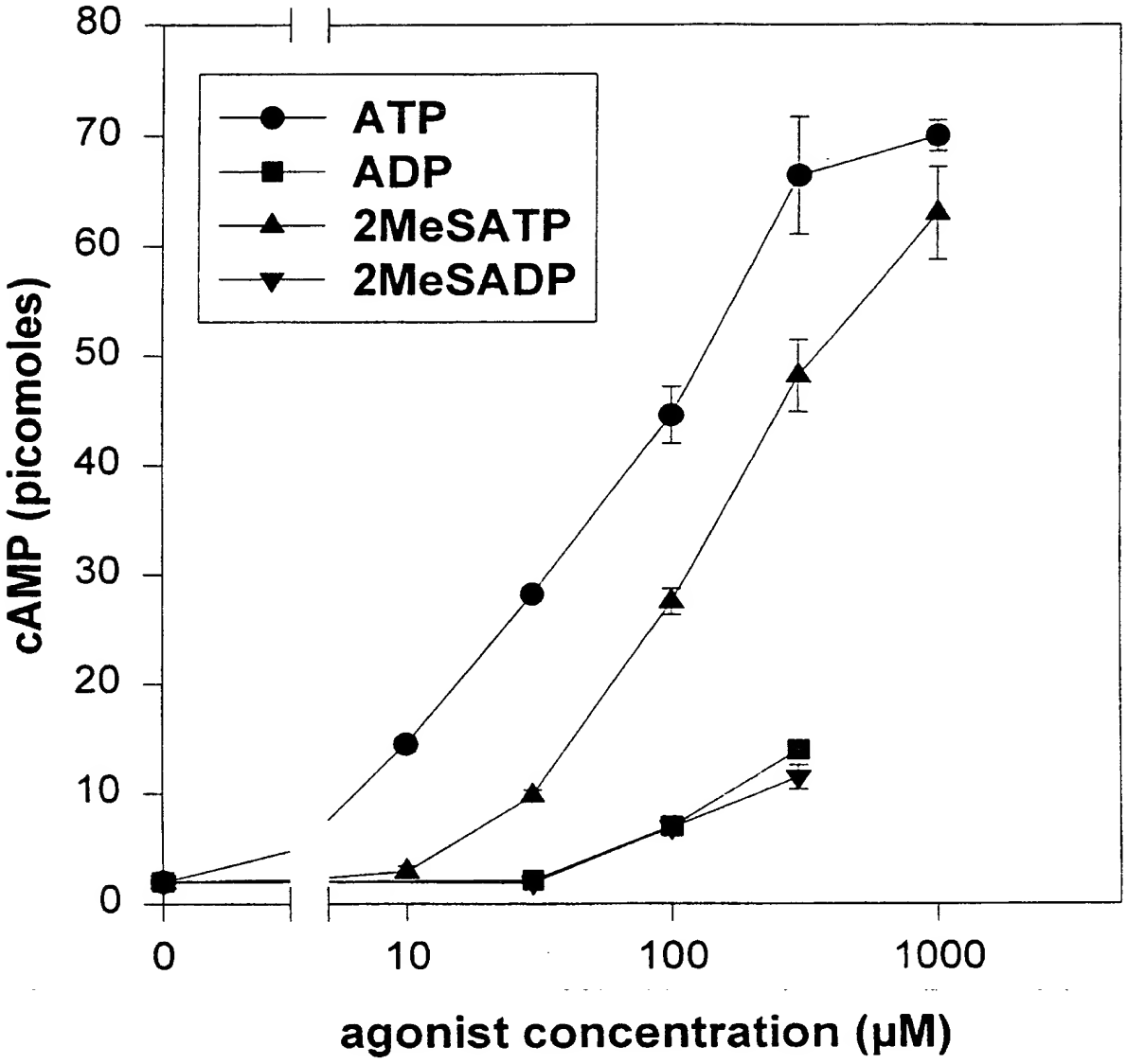


FIG.5

INTERNATIONAL SEARCH REPORT

Int Application No
PCT/BE 98/00108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C07K14/705 C07K16/28 C12Q1/68
G01N33/50 G01N33/566 A61K31/70 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE GENBANK Accession No. AA321112, 19 April 1997 ADAMS M.D. ET AL.: "EST 23640, H. sapiens cDNA similar to S. cerevisiae YHR066w (SSF1)" XP002083514 compare nt 1-312 with nt 340-650 of seq. ID 1 ---	1,2, 7-17,19, 20
A	WO 97 20045 A (COR THERAPEUTICS INC) 5 June 1997 see abstract see page 15, line 21 - page 29, line 9 --- -/--	1-68

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 November 1998	23/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 98/00108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COMMUNI D. & BOEYNAEMS J.M.: "Receptors responsive to extracellular pyrimidine nucleotides" TRENDS IN PHARM. SCI., vol. 18, March 1997, pages 83-86, XP002083512 see the whole document	1-68
P,X	COMMUNI D. ET AL.: "Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenyl cyclase." J. BIOL. CHEM., vol. 272, no. 51, 19 December 1997, pages 31969-31973, XP002083513 see the whole document	1-20, 42-44, 48,50, 52,56,67

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BE 98/00108

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
The ligands, antiligands, agonists and antagonists mentioned in claims 24-26, 35, 46, 47, 54, 55, 61, 62, 64, 65, 68 are not sufficiently disclosed in the description to allow a meaningful and complete search of the subject.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/BE 98/00108

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9720045 A	05-06-1997	AU 1075397 A	19-06-1997
		EP 0868510 A	07-10-1998